

In the Claims:

Please amend the claims as follows:

Claims 1 – 96 (Canceled).

Claim 97. (CURRENTLY AMENDED) A new method for the quantification of ligand

binding to a surface, using hapten-conjugated ligands, comprising:

[a] applying a hapten-ligand, comprising a ligand possessing an antibody-recognizable hapten, to said surface, and,

[b] waiting for a period of time, so as to allow a binding of said hapten-ligand to said surface, thereby producing bound ligand, and,

[c] removing any unbound hapten-ligand, from said surface, and,

[d] solubilizing said bound ligand, thereby producing a lysate, and,

[e] applying separately onto a membrane,

[1] said lysate, and,

[2] a plurality of standards, comprising solutions containing increasing levels of known amounts of the hapten-ligand,

thereby producing membrane-bound hapten-ligand and,

[f] applying to said membrane,

[1] an enzyme-conjugated antibody ~~to~~ specific for said hapten, and,

[2] a color or light-producing substrate that contacts the enzyme on said enzyme-conjugated antibody ~~for said enzyme~~,

thereby producing a signal, and,

[g] comparing said signal arising from said enzyme associated with said

membrane-bound hapten-ligand arising from said standards, to the known amount of hapten-ligand contained in said standards, thereby producing a standard curve, and,

[h] comparing said signal arising from said enzyme associated with said membrane-bound hapten-ligand arising from said lysate, to said standard curve, thereby quantifying the amount of the hapten-ligand contained in said membrane-bound hapten-ligand arising from said lysate,

whereby the quantifying of the amount of the membrane-bound hapten-ligand arising from said lysate, is used to quantify the amount of said hapten-ligand originally bound to said surface, and, whereby the use of radio- labeled ligand is avoided.

Claim 98. (CURRENTLY AMENDED) The method of Claim 97 step [a], wherein said

ligand being applied to said surface ~~in step 97[a]~~, is comprising:

[a] said hapten-ligand, and,

[b] un-conjugated ligand, comprising said ligand which does not possess said hapten, and,

whereby said signal from Claim 97 step [f] step [2] arising from said lysate containing both said hapten-ligand, and said un-conjugated ligand is compared to said signal arising from said lysate containing said hapten-ligand only, thereby verifying the specific binding of said hapten-ligand to said surface.

Claim 99. (CURRENTLY AMENDED) The method of Claim 97 step [e], wherein the producing of said membrane-bound hapten-ligand ~~of step 97[e]~~ is further ~~comprising~~ comprises:

[a] ~~the separating of~~ said lysate by electrophoresis, and,

[b] ~~the separating of~~ said standards, by electrophoresis, and,

[b] ~~the applying of~~ separated lysate, and separated standards to said membrane support, and,

whereby the location of said signal from Claim 97 step [f] step [2] arising from said hapten-ligand, contained in separated lysates, on said membrane, is verified by comparing its said signal it to the location of said signal arising from separated standards, on said membrane.

Claim 100. (PREVIOUSLY ADDED) The method of Claim 99 wherein said method of electrophoresis is selected from the group consisting of SDS-PAGE, electrophoresis according to Schagger Von Jagow, and agarose electrophoresis.

Claim 101. (CURRENTLY AMENDED) The method of Claim 97 step [f] step [1], wherein ~~said~~ the applying of an antibody to said membrane support is further comprising:

[a] applying an anti-hapten antibody, comprising an antibody specific for said hapten, to said membrane support and,

[b] applying an enzyme-conjugated antibody specific for said ~~to~~ anti-hapten antibody, to said membrane support.

~~whereby the sensitive detecting of said membrane-bound hapten-
ligand is afforded by the use of said antibodies.~~

Claim 102. (PREVIOUSLY ADDED) The method of Claim 97, wherein said surface includes biological cells.

Claim 103. (PREVIOUSLY ADDED) The method of Claim 97, wherein said hapten includes compounds which can be specifically recognized by an antibody.

Claim 104. (PREVIOUSLY ADDED) The method of Claim 97, wherein said hapten is selected from the group consisting of, fluorescein, biotin, rhodamine, and digoxigenin.

Claim 105. (PREVIOUSLY ADDED) The method of Claim 97, wherein said ligand is a biological factor.

Claim 106. (PREVIOUSLY ADDED) The method of Claim 97, wherein said ligand is a protein.

Claim 107. (CURRENTLY AMENDED) The method of Claim 97 106, wherein said protein is selected from the group consisting of transferrin, concanavalin A, avidin, annexin V, and insulin.

Claim 108. (PREVIOUSLY ADDED) The method of Claim 97, wherein said ligand is DNA.

Claim 109. (CURRENTLY AMENDED) The method of Claim 97 step [e], wherein said ~~applying onto a membrane method includes blotting~~ the applying of said lysate and said standards onto said membrane is achieved by a blotting method.

Claim 110. (CURRENTLY AMENDED) The method of Claim ~~108~~ 109, wherein said blotting method is selected from the group consisting of electroblotting, dot blotting, slot blotting, or Western blotting.

Claim 111. (CURRENTLY AMENDED) The method of Claim 97 step [e], wherein said membrane ~~support~~ includes ~~conventional~~ transfer membranes.

Claim 112. (CURRENTLY AMENDED) The method of Claim 97 step [e], wherein said membrane ~~support~~ is selected from the group consisting of protein binding membranes, and DNA binding membranes.

Claim 113. (CURRENTLY AMENDED) The method of Claim 97 step [e], wherein said membrane ~~support~~ is selected from the group consisting of nitrocellulose, or nytran.

Claim 114. (PREVIOUSLY ADDED) The method of Claim 97 step [f], wherein said enzyme is horseradish peroxidase.

Claim 115. (CURRENTLY AMENDED) The method of claim 97 step [f] step [2], wherein the quantifying of said signal, arising from said light producing substrate, on said membrane ~~support~~, is further comprising:

- [a] placing said membrane ~~support~~ in contact with photographic film, and,
- [b] analyzing said signal on said photographic film using an imager.

Claim 116. (NEW) The method of claim 97 step [f], wherein the applying to said membrane further comprises an initial applying to said membrane of a block solution, comprising a solution containing agents which occupy all non-occupied sites on said membrane, thereby preventing the binding of the antibody specific for said hapten, to sites other than said membrane-bound hapten-ligand.